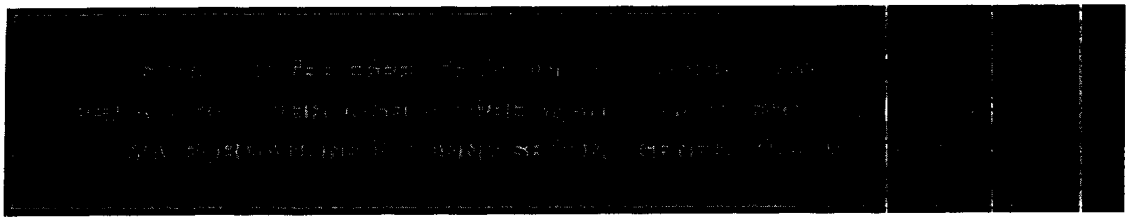
Appendix 10. 5

CONFIDENTIAL

STUDY REPORT



PROTOCOL CODE: INGREDIA-13-2/0501/ Pathoanatomy-F2

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STUDY IN CONFORMITY WITH THE GOOD ANIMAL EXPERIMENT PRACTICES

OCTOBER 2001

EXPERIMENT PERIOD: JULY - AUGUST 2001

CONTENTS

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STUDY OF THE EFFECTS OF "ING 911" HYDROLYSATE GIVEN TO FEMALE RATS THROUGHOUT PREGNANCY, ON INTERNAL AND EXTERNAL MALFORMATIONS IN YOUNG SECOND-GENERATION WISTAR RATS

AUTHOR

I, undersigned, declare that the work described in this report was completed under my responsibility and that this report accurately corresponds to the results obtained.
Moreover, I declare that the present study was carried out in accordance with the Standard Work Protocols of the Pathoanatomy Laboratory of the Faculty of Medicine of Nancy, H. Poincaré University and in accordance with the Good Laboratory Practices, including original document filing.

Vandoeuvre-lès-Nancy, October 25th, 2001

Professor F. PLENAT

1 – INTRODUCTION

At the INGREDIA's request, the Pathoanatomy Laboratory of the Faculty of Medicine of Nancy, H. Poincaré University, carried out a macroscopic and microscopic study in order to evaluate the anatomical effects of "ING 911" hydrolysate, given to female rats from Wistar strain throughout pregnancy, on internal and external malformations in young second-generation males and females.

Eight first-generation females, born from control females having received 150 mg/kg/d of powdered skim milk throughout pregnancy and eight others, born from females treated with the 150 mg/kg daily dose of ING 911 hydrolysate for the same time, were fertilised by different non-consanguineous males, born from females of the same treatment groups.

At birth, the litters were reduced to 8 youngs (4-4 or 3-5, depending on the number of males and females in the litters). In every litter, a male and a female, aged 18 or 19 days, were randomly selected and transferred to the Pathoanatomy Laboratory of the Faculty of Medicine of Nancy to carry out macroscopic and histological axaminations in order to detect any possible internal and external malformations

2 - MATERIALS AND METHODS

2.1 - Animals

Sixteen Wistar females of first generation, originating from the litters of the main behavioural toxicology study, were used. After crossing with non-consanguineous males of first generation, born from females of same treatment groups, the fertilised females were installed in type-F polycarbonate cages (48 x 27 x 20 cm, U.A.R., 91 - Epinay-Sur-Orge, France). At birth, after recording of their size, the litters were reduced to 8 youngs (4-4 or 3-5), in order to study comparable-sized groups. The young of every litter were marked subcutaneously with a vital dye (alcyan blue) to be individualised. Following hair growth, a definitive marking, with picric acid, was applied to the hairs according to a given code. The females and their youngs were housed in a air-conditioned animal care facility, at a temperature of 22-24°C. The females had food (dry food M25, Ets Piétrement, 77-Provins, France) and drink *ad libitum* and were subjected to a light-dark cycle of 12 hours. All the rats of the various groups were treated similarly and under the same conditions.

Crosses, pregnancy follow-up, and litter housing for three weeks following birth were carried out within the Centre de Recherches ETAP-Ethologie Appliquée 13, rue du Bois de la Champelle - F - 54500 Vandoeuvre-lès-Nancy (Dr. A. NEJDI).

2.2 - Experimental procedure

At the age of 18 or 19 days, 8 males and 7 females of the "Treatment" group and 8 males and 8 females of the "Control" group were randomly selected at a rate of one male and one female in every litter and transferred to the Pathoanatomy Laboratory of the Faculty of Medicine of Nancy, for macroscopic and histological examinations to detect any possible external and internal general malformations.

Variables studied:

- external general malformations;
- internal visceral malformations;
- histological examinations.

3 - RESULTS

Table 1

GENERAL MALFORMATIONS						
Litter Code	C1		C5		C6	
Birth date	19-07-01		19-07-01		20-07-01	
Control group	Male No. 2 (D6485)	Female No. 4 (D6488)	Male No. 3 (D6486)	Female No. 1 (D6491)	Male No. 1 (D6487)	Female No. 2 (D6490)
Head						
- normal	YES	YES	YES	YES	YES	YES
Eyes						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Ears						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Mouth						
- normal	YES	YES	YES	YES	YES	YES
- cleft lip	NOT	NOT	NOT	NOT	NOT	NOT
- cleft palate	NOT	NOT	NOT	NOT	NOT	NOT
Foreleg						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES
Rear legs						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES
Anus						
- normal	YES	YES	YES	YES	YES	YES
Ext. genit. organs						
- normal	YES	YES	YES	YES	YES	YES
Tail						
- normal	YES	YES	YES	YES	YES	YES
- size						
Agenesis	0	0	0	0	0	0
Ribs						
- number	12	12	12	12	12	12
- layout	everything OK	everything OK	everything OK	everything OK	everything OK	everything OK
VISCERAL MALFORMATIONS						
Heart						
- normal	YES	YES	YES	YES	YES	YES
- cavities	everything OK	everything OK	everything OK	everything OK	everything OK	everything OK
Lungs						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Kidneys						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Digestive system						
Liver	G-/S+	G-/S+	G-/S-	G-/S+	G-/S-	G-/S+
Stomach	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
Intestines	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL

Table 2

GENERAL MALFORMATIONS						
Litter Code **Birth date**	**C7** **20-07-01**		**C10** **21-07-01**		**C11** **21-07-01**	
Control group	Male No. 1 (D6484)	Female No. 3 (D6489)	Female (*) No. 2 (D6505)	Female No. 3 (D6501)	Male No. 3 (D6506)	Female No. 1 (D6502)
Head - normal	YES	YES	YES	YES	YES	YES
Eyes - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Ears - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Mouth - normal - cleft lip - cleft palate	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT
Foreleg - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Rear legs - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Anus - normal	YES	YES	YES	YES	YES	YES
Ext. genit. organs - normal	YES	YES	YES	YES	YES	YES
Tail - normal - size	YES	YES	YES	YES	YES	YES
Agenesis	0	0	0	0	0	0
Ribs - number - layout	12 everything OK	12 everything OK	12 everything OK	12 everything OK	12 everything OK	12 everything OK
VISCERAL MALFORMATIONS						
Heart - normal - cavities	YES everything OK	YES everything OK	YES everything OK	YES everything OK	YES everything OK	YES everything OK
Lungs - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Kidneys - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Digestive system Liver Stomach Intestines	G-/S- NORMAL NORMAL	G-/S+ NORMAL NORMAL	G+/S- NORMAL NORMAL	G-/S- NORMAL NORMAL	G+/S- NORMAL NORMAL	G+/S- NORMAL NORMAL

(*) In the litter C10, the male No. 2 proved to be a female.

Table 3

GENERAL MALFORMATIONS				
Litter Code **Birth date**	**C12** **21-07-01**		**C13** **21-07-01**	
Control group	Male No. 3 (D6507)	Female No. 1 (D6503)	Female No. 3 (D6508)	Female No. 1 (D6504)
Head - normal	YES	YES	YES	YES
Eyes - normal - number	YES 2	YES 2	YES 2	YES 2
Ears - normal - number	YES 2	YES 2	YES 2	YES 2
Mouth - normal - cleft lip - cleft palate	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT
Foreleg - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Rear legs - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Anus - normal	YES	YES	YES	YES
Ext. genit. organs - normal	YES	YES	YES	YES
Tail - normal - size	YES	YES	YES	YES
Agenesis	0	0	0	0
Ribs - number - layout	12 everything OK	12 everything OK	12 everything OK	12 everything OK
VISCERAL MALFORMATIONS				
Heart - normal - cavities	YES everything OK	YES everything OK	YES everything OK	YES everything OK
Lungs - normal - number	YES 2	YES 2	YES 2	YES 2
Kidneys - normal - number	YES 2	YES 2	YES 2	YES 2
Digestive system Liver Stomach Intestines	G-/S- NORMAL NORMAL	G+/S- NORMAL NORMAL	G+/S- NORMAL NORMAL	G-/S- NORMAL NORMAL

Table 4

GENERAL MALFORMATIONS						
Litter Code **Birth date**	**C2** **19-07-01**		**C3** **19-07-01**		**C4** **19-07-01**	
Control group	Male No. 3 (D6496)	Female No. 1 (D6492)	Male No. 2 (D6497)	Female No. 4 (D6493)	Male No. 1 (D6498)	Female No. 1
Head - *normal*	YES	YES	YES	YES	YES	-
Eyes - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	-
Ears - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	-
Mouth - normal - cleft lip - cleft palate	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT	-
Foreleg - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	-
Rear legs - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	-
Anus - normal	YES	YES	YES	YES	YES	-
Ext. genit. organs - *normal*	YES	YES	YES	YES	YES	-
Tail - normal - size	YES	YES	YES	YES	YES	-
Agenesis	0	0	0	0	0	-
Ribs - number - layout	12 everything OK	12 everything OK	12 everything OK	12 everything OK	12 everything OK	-
VISCERAL MALFORMATIONS						
Heart - normal - cavities	YES everything OK	YES everything OK	YES everything OK	YES everything OK	YES everything OK	-
Lungs - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	-
Kidneys - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	-
Digestive system Liver Stomach Intestines	G+/S- NORMAL NORMAL	G+/S- NORMAL NORMAL	G+/S- NORMAL NORMAL	G+/S- NORMAL NORMAL	G-/S+ NORMAL NORMAL	-

Table 5

GENERAL MALFORMATIONS						
Litter Code **Birth date**	**C8** **21-07-01**		**C9** **21-07-01**		**C15** **22-07-01**	
Control group	Male No. 4 (D6499)	Female No. 2 (D6495)	Male No. 4 (D6500)	Female No. 1 (D6494)	Male No. 1 (D6509)	Female No. 2 (D6512)
Head - normal	YES	YES	YES	YES	YES	YES
Eyes - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Ears - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Mouth - normal - cleft lip - cleft palate	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT
Foreleg - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Rear legs - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Anus - normal	YES	YES	YES	YES	YES	YES
Ext. genit. organs - normal	YES	YES	YES	YES	YES	YES
Tail - normal - size	YES	YES	YES	YES	YES	YES
Agenesis	0	0	0	0	0	0
Ribs - number - layout	12 everything OK	12 everything OK	12 everything OK	12 everything OK	12 everything OK	12 everything OK
VISCERAL MALFORMATIONS						
Heart - normal - cavities	YES everything OK	YES everything OK	YES everything OK	YES everything OK	YES everything OK	YES everything OK
Lungs - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Kidneys - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Digestive system Liver Stomach Intestines	G-/S+ NORMAL NORMAL	G+/S- NORMAL NORMAL	G-/S+ NORMAL NORMAL	G+/S- NORMAL NORMAL	G+/S+ NORMAL NORMAL	G-/S+ NORMAL NORMAL

Table 6

GENERAL MALFORMATIONS				
Litter Code **Birth date**	**C16** **22-07-01**		**C18** **22-07-01**	
Control group	Male No. 3 (D6510)	Female No. 4 (D6513)	Female No. 2 (D6511)	Female No. 1 (D6514)
Head - normal	YES	YES	YES	YES
Eyes - normal - number	YES 2	YES 2	YES 2	YES 2
Ears - normal - number	YES 2	YES 2	YES 2	YES 2
Mouth - normal - cleft lip - cleft palate	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT
Foreleg - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Rear legs - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Anus - normal	YES	YES	YES	YES
Ext. genit. organs - normal	YES	YES	YES	YES
Tail - normal - size	YES	YES	YES	YES
Agenesis	0	0	0	0
Ribs - number - layout	12 everything OK	12 everything OK	12 everything OK	12 everything OK
VISCERAL MALFORMATIONS				
Heart - normal - cavities	YES everything OK	YES everything OK	YES everything OK	YES everything OK
Lungs - normal - number	YES 2	YES 2	YES 2	YES 2
Kidneys - normal - number	YES 2	YES 2	YES 2	YES 2
Digestive system Liver Stomach Intestines	G-/S+ NORMAL NORMAL	G-/S+ NORMAL NORMAL	G-/S+ NORMAL NORMAL	G-/S+ NORMAL NORMAL

4 – CONCLUSION

No malformation was observed during examination under operating microscope in the young second-generation male and female rats of both treatment groups.

The systematic histological controls (lungs, heart, liver, kidneys, pancreas, stomach, colon and small intestine) appeared normal. However, a very discrete macrovesicular steatosis (named G+ in tables) was observed in some animals of both "Treatment" and "Control" groups. This steatosis is generally, but not constantly, observed in animals whose liver glycogen storage is reduced. That could be explained by the fact why these animals were sacrificed after a 16-hour fast.

In conclusion, the daily administration of "ING 911" hydrolysate, at the oral dose of 150 mg/kg, in female Wistar rats throughout their pregnancy, does not induce general or visceral malformations in the young second-generation males and females.

Vandoeuvre-lès-Nancy, October 25th, 2001

Professor F. PLENAT

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